The Contamination Level in the NTP Anthraquinone Bioassay was 0.6% and Not 0.1% as Reported in the Abstract of Technical Report 494

Submitted to
The National Toxicology Program
National Institutes of Health

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February 2, 2004

Summary

The Abstract of TR 494 of the National Toxicology Program (NTP) on the carcinogenicity of anthraquinone (AQ) states that the level of contamination of the test material by GC analysis was 0.1%. HPLC analytical studies in that same report, however, noted a contamination level of 0.5% including unidentified compound(s).

Because of that GC vs. HPLC inconsistency, and because of the discrepancy that the AQ bioassay material has been shown to contain contaminating mutagenic activity that may not all be assigned to the primary contaminant, 9-nitroanthracene (9-NA), Arkion Life Sciences undertook a state-of-the-art analysis of the bioassay material. This new analysis shows that the contamination level of the AQ bioassay material was actually 0.6 %. Contaminants included 9-nitroanthracene, polycyclic aromatic hydrocarbons, and other unidentified organic and nitro-organic compounds. The presence of these chemicals is consistent with the fact that this material was produced by the oxidation of anthracene derived from coal tar.

These data indicate that the level of contamination is actually 6-fold greater than is stated in the Abstract, and suggests that the mutagenic contamination resides with compounds in addition to 9-NA. These observations strengthen the case that it is plausible that all of the tumor induction in the NTP bioassay may be assigned to mutagenic and carcinogenic contaminants.

Background

This is a supplemental communication from Arkion Life Sciences (Formerly Environmental Biocontrol, Intl.) regarding the NTP AQ Bioassay (NTP, 2004) (see also the Adalsteinsson Submission to NTP of January 8, 2004). The central issue of concern is that the test material used in the NTP cancer bioassay was mutagenic in the Ames test bacterial strains TA98, TA100, and TA1537 (Butterworth *et al.*, 2001). Removal of the primary contaminant 9-nitroanthracene (9-NA) and other contaminating organics by recrystalization resulted in the complete loss of mutagenic activity (Butterworth *et al.*, 2001). The degree of this contamination was of a magnitude that confounded interpretation of the bioassay (Butterworth *et al.*, 2001).

9-NA was found at 0.12% in the bioassay material (Butterworth *et al.*, 2001). Although other organics were present, the fact that nitroaromatic compounds often exhibit strong

mutagenic and carcinogenic activity suggested that 9-NA was the likely bad actor. Thus, the strength of the argument that the bioassay was flawed was based on the degree of mutagenic activity and no further analytical work was done at that time.

NTP Approach to the Contamination Problem

Members of the NTP Technical Reports Review Subcommittee who reviewed the original draft of the AQ bioassays (NTP, 1999) were unaware that there was a contamination problem and approved the report. To address the concerns raised in the Butterworth et al. paper, the NTP withheld release of the original AQ carcinogenesis report, and began their own analytical and mutagenicity evaluations, which are incorporated in the current draft report (NTP, 2004). The question was raised whether the mutagenic activity of 9-NA alone was of sufficient potency to account for the degree of activity seen in the bioassay material. It was possible that the observed contaminating mutagenic and potential carcinogenic activity might reside with more than just the 9-NA. However, this would mean that the degree of contamination would have to be greater than 0.1% as reported in the Abstract of TR 494. Therefore, in the past weeks Arkion Life Sciences has undertaken an extensive, state-of-the-art analytical reevaluation of the AQ bioassay material.

New Analytical Studies

GC analysis can often fail to detect substantial contamination with low levels of multiple contaminants because the minimum amount of material is applied to the column to avoid overloading. The GC/FID analysis of the NTP bioassay material indicated a contaminant level of 0.1% (NTP, 2004 - p. J-2) and is so reported in the Abstract of the draft report. However when the same material was evaluated using HPLC/UV analysis, a contaminant level of 0.5% was seen with two impurities of 0.3% and 0.2% relative to the AQ peak (NTP, 2004 - p. J-2). The greater peak was identified as 9-NA. The second peak was not identified. This information is not presented in the Abstract of the study report (NTP, 2004).

Much improved analysis can be conducted if the contaminants can be removed and studied separately from the main material. In the Arkion studies, after the AQ had been removed by recrystalization, the remaining supernatant was quantitatively analyzed for contaminants. The results of this new analysis revealed that the contamination level in the AQ bioassay material was actually 0.6% (Mathre, 2004). Classes of contaminants found are noted below. Identification of individual components is an ongoing longer-term project.

Contaminants in the AQ Bioassay Material	
0.12%	9-nitroanthracene
0.05%	polycyclic aromatic hydrocarbons
0.45%	unidentified organics and other nitro-organics
0.62%	Total

Implications of the New Analytical Studies

The AQ preparation used in the NTP bioassays was more contaminated than had been previously acknowledged. The report of a 0.1% contamination level in the Abstract of TR 494 is incorrect and misleading. The actual 6-fold higher level of contamination indicates that the contaminating mutagenic activity probably resides with more than just the 9-NA component. It

should be noted that as a class nitroaromatic compounds often exhibit potent mutagenic and carcinogenic activity.

The procedure employed to make the anthraquinone used in the NTP bioassay involves oxidation of anthracene isolated from coal tar. Such preparations are often contaminated with polycyclic aromatic hydrocarbons and nitroaromatic compounds. The Arkion analysis of the NTP AQ bioassay sample is consistent with that history. AQ from that process is neither used nor imported into the United States.

Conclusion and Recommended Course of Action

The new analytical data summarized here strengthen the case that it is plausible that the tumor induction in the NTP AQ bioassay was produced by the contaminating material. The weight of evidence indicates that no conclusions as to the carcinogenic activity of AQ can be drawn from the bioassays that were run. This flawed study does not meet the high standards set by the NTP in the performance of cancer bioassays. AQ is an important compound in commerce and it is vital that we have a quantitative understanding of its carcinogenic potential in order to make sound decisions on acceptable exposures. The only avenue to gain this information is to conduct a new bioassay using the uncontaminated, non-anthracene based AQ in common use today. We urge that the current AQ draft report be withdrawn and that the NTP conduct a new bioassay as soon as is practical.

References

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